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FROM: KATHRYN LAPPEGARD

RE: U.S. PATENT APPLICATION SERIAL NO. 09/020,716
ATTORNEY DOCKET NO. 0815AAAE

DATE: 03/17/05 FAX NUMBER: (703) 872-9306

NUMBER OF PAGES FOLLOWING THIS SHEET: 77

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TRANSMITTAL FORM

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Total Number of Pages in This Submission	76

Application Number	09/020,716
Filing Date	02/09/1998
First Named Inventor	Rudolf Jung
Art Unit	1638
Examiner Name	McElwain, Elizabeth F.
Attorney Docket Number	0815AAAE

ENCLOSURES *(check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment / Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
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- ☐ CD, Number of CD(s) ____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☒ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) *(please identify below)*:
  1) Transmittal of Appellant's Brief

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm	Pioneer Hi-Bred International, Inc.		
Signature	Kathryn K. Lappegard		
Printed Name	Kathryn K. Lappegard		
Date	March 17, 2005	Reg. No.	46,857

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Attorney Docket No. 0815AAAE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Jung et al.
Application No.	:	09/020,716
Filed	:	02/09/1998
Group Art Unit	:	1638
Examiner	:	McElwain, Elizabeth F.
For	:	Alteration of Amino Acid Compositions in Seeds

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Alexandria, VA 22313-1450

TRANSMITTAL OF APPEAL BREIF
(PATENT APPLICATION – 37 CFR § 1.192)

1. Transmitted herewith, in triplicate, is the Appeal Brief in this application, with respect to the Notice of Appeal filed on January 25, 2005.

2. STATUS OF APPLICANT

This application is on behalf of

- ☒ other than a small entity.
- ☐ a small entity

A statement:

- ☐ is attached.
- ☐ was already filed.

3. FEE FOR FILING APPEAL BRIEF

Pursuant to 37 CFR § 41.20(b)(2) the fee for filing the Appeal Brief by other than a small entity is $500.00.

Attorney Docket No. 0815AAAE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No. 09/020,716
Transmittal of Appeal Brief Dated 03/17/2005

4. EXTENSION OF TERM

The proceedings herein are for a patent application and the provisions of 37 CFR § 1.136 apply.

(complete (a) or (b), as applicable)

(a) ☐ Applicant petitions for an extension of time under 37 CFR § 1.136 for the total number of months checked below:

Extension of time requested for:

☐	1 Month (37 CFR 1.17(a)(1))	$ 110.00
☐	2 Months (37 CFR 1.17(a)(2))	$ 430.00
☐	3 Months (37 CFR 1.17(a)(3))	$ 980.00
☐	4 Months (if available) (37 CFR 1.17(a)(4))	$1,530.00
☐	5 Months (if available) (37 CFR 1.17(a)(5))	$2,080.00

If an additional extension of time is required, please consider this a petition therefor.

(check and complete the next item if applicable)

☐ An extension for _____ months has already been secured and the fee paid therefor of $_____ is deducted from the total fee due for the total months of extension now requested.

or

(b) ☒ Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

5. TOTAL FEE DUE

The total fee due is:

Appeal Brief Fee $500.00

Extension Fee (if any) $_____

TOTAL FEE DUE $500.00

6. FEE PAYMENT

Authorization is hereby made to charge the amount of $500.00 to Deposit Account No. 16-1852.

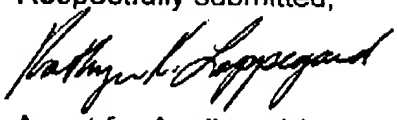
Charge any additional fees which may be required by this paper or credit any overpayment to Deposit Account No. 16-1852.

A duplicate copy of this sheet is attached.

7. FEE DEFICIENCY

If any additional extension and/or fee is required, and/or if any additional fee for claims is required, charge Deposit Account No. 16-1852.

Respectfully submitted,



Agent for Applicant(s)
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APPELLANT'S BRIEF (37 C.F.R. § 1.192)

This brief is in furtherance of the Notice of Appeal, filed in this case on January 25, 2005.

The fees required under § 1.17, and any required petition for extension of time for filing this brief and fees therefor, are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief is transmitted in triplicate. (37 C.F.R. § 1.192(a).

This brief contains these items under the following headings, and in the order set forth below (37 C.F.R. § 1.192(c)).

I REAL PARTY OF INTEREST
II RELATED APPEALS AND INTERFERENCES
III STATUS OF CLAIMS
IV STATUS OF AMENDMENTS
V SUMMARY OF INVENTION

VI ISSUES

VII GROUPING OF CLAIMS

VIII ARGUMENTS

(check each category of ARGUMENT submitted in this brief)

- ☒ ARGUMENT: VIIIA REJECTIONS UNDER 35 USC 112, FIRST PARAGRAPH
- ☒ ARGUMENT: VIIIB REJECTIONS UNDER 35 USC 112, SECOND PARAGRAPH
- ☒ ARGUMENT: VIIIC REJECTIONS UNDER 35 USC 102
- ☒ ARGUMENT: VIIID REJECTIONS UNDER 35 USC 103
- ☐ ARGUMENT: VIIIE REJECTIONS OTHER THAN 35 USC 102, 103, AND 112

IX APPENDIX OF CLAIMS INVOLVED IN THE APPEAL

☐ OTHER MATERIALS THAT APPELLANT CONSIDERS NECESSARY OR DESIRABLE

The final page of this brief bears the practitioner's signature.

I REAL PARTY IN INTEREST

The subject application is owned by Pioneer Hi-Bred International, Inc. of Johnston, Iowa.

II RELATED APPEALS AND INTERFERENCES

With respect to other appeals or interferences that will directly affect, or be directly affected by, or have a bearing on the Board's decision in this appeal, there are no such appeals or interferences.

III STATUS OF CLAIMS

The status of the claims in this application are:

A. TOTAL NUMBER OF CLAIMS IN APPLICATION

Claims in the application are: 1-120

B. STATUS OF ALL THE CLAIMS

1. Claims cancelled: 1-78, 80-89, 92-104, 106-111, and 117-120
2. Claims withdrawn from consideration but not cancelled: None
3. Claims pending: 79, 90, 91, 105, and 112-116
4. Claims allowed: None
5. Claims rejected: 79, 90, 91, 105, 112-116

C. CLAIMS ON APPEAL

The claims on appeal are: 79, 90, 91, 105, and 112-116

IV STATUS OF AMENDMENTS

Insofar as understood by the appellant, the status of the amendment filed January 25, 2005, has not been acted upon by the Examiner nor entered.

V SUMMARY OF INVENTION

The present invention provides methods for increasing the level of lysine, sulfur-containing amino acids or both, in a cereal plant. Specification page 4, lines 5-19. The invention employs a promoter with endosperm-preferred expression. Specification page 4, line 20 to specification, page 5, line 7. The promoter is linked to a barley alpha-hordothionin polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid. Specification page 6, lines 21 to page 7 line 4 and specification page 8, line 3 to page 10, line 20. Also provided are expression cassettes and plant seed produced by such method. Specification page 4, lines 5-6 and page 5, lines 1-12.

Crop plants in feed formulations must typically be supplemented with specific amino acids in order to provide animals consuming the plants with essential nutrients necessary for their growth. Lysine, methionine and threonine are among the amino acids necessary for animal nutrition. Various attempts have been made to increase the level of these amino acids, which attempts have been met with limited success. See the specification, page 1.

One means of enhancing levels of amino acid levels of crop plants is modification of amino acid biosynthesis in the plant. In the approach of protein sequence modification, a gene is identified encoding a major protein as the target for modification to contain more codons of essential amino acids. Specification, page 2, lines 1-2 and lines 22-23. An important approach is to select a region of the protein that can be modified without affecting the overall structure, stability, function and other cellular and nutritional properties of the protein. Specification page 2, line 22 to page 3, line 3. In one example of attempts to achieve this goal to date, expression of the *lysC* gene resulted in increase methionine and threonine biosynthesis, but only a 6-7% increase in total threonine or methionine in seed. Specification, page 2, lines 10-19. Use of sulfur-rich Brazil Nut Protein increased methionine content, but decreased lysine content and further has been identified as a major food allergen. Specification, page 3, lines 14-23. Therefore there is a need to improve the nutritional value of plant seeds without accompanying detrimental side affects. Specification page 4, lines 1-3.

VI ISSUES

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

Claim 103 is rejected under 35 U.S.C. §102(b) as being anticipated by Powell et al, U.S. Patent No. 3,909,288.

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §103 as being obvious over Rao et al., U.S. patent 5,885,802 "in view of Applicants' admission."

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §103 as being obvious over Rao et al., U.S. patent 5,990,389 "in view of Applicants' admission."

VII GROUPING OF CLAIMS

The claims do not stand or fall together except as regards the second paragraph section 112 second paragraph rejection. The patentability of the claims will be argued separately.

VIIIA ARGUMENTS – Rejections Under 35 U.S.C. § 112, First Paragraph

Rejection of Claims 79, 90, 91, 103, 105 and 112-116

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. The Examiner states the specification teaches a transformed cereal plant seed having an elevated lysine, methionine and cysteine content (about 10% to about 35% by weight compared to untransformed cereal plant seed) comprising the modified hordothionin gene of SEQ ID NO: 2 (HT-12), vectors, plant cells and transformed plants comprising said modified hordothionin gene. The Examiner finds the specification does not teach the range of 7 mole% to 50 mole% of lysine or 6 mole % to 40 mole% of sulfur-containing amino acids for any seed protein, and further does not teach any seed proteins having both of these ranges. The Examiner says that working examples need to be provided, and there is an absence of guidance in identifying such sequences and transforming into plants.

In response to the rejection, the Applicant pointed out that the specification includes a number of examples of proteins useful in the invention and teaches one how to identify such proteins. The Examiner says that the example of a construct of HT-12 and an endosperm preferred promoter is not adequate. A declaration was provided by Dr. Rudolph Jung demonstrating production of proteins of the invention using a construct with sequences encoding modified hordothionin HT-12 as well as soybean 2S albumin (ESA) as compared with a control. The Examiner responded that one working example is not sufficient and "the example provided in the Declaration of Jung does not provide information to support the claims."

Claim 103 was cancelled in the amendment after of January 25, 2005. Since the amendment complies with the Examiner's request, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

The claims recite a method, cereal plant seed, or cassette comprising a modified barley alpha-hordothionin polynucleotide, operably linked to a seed endosperm-preferred promoter, which nucleotide encodes a barley alpha-hordothionin protein modified to contain about 7 mole %to about 40 mole % lysine or 6 mole % to about 40 mole % of a sulfur-containing amino acid, resulting in increased levels of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

The Examiner's primary issue, as outlined in the last Office Action, appears to be directed to the "lack of working examples of protein coding sequences having the claimed ranges of mole % lysine and sulfur containing amino acids" and identifying and transforming such sequences. The Examiner focuses on the "one" example. However, the Applicant has demonstrated that there are various methods to identify proteins to be used in the invention, listed various sequences encoding proteins useful in the invention, and described methodology by which to identify the proteins. Compliance with the enablement requirement of section 112 "does not turn on whether an example is disclosed. All that is required by the law is an objective enablement of the invention, whether by working examples or by the use of broad

terminology." *In re Vaeck*, 947 F2d 488, 496 (Fed. Cir. 1991). Eight considerations are indicated in *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988) in determining if undue experimentation is necessary to practice an invention. These are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence of working examples of the invention, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims.

When determining the quantity of experimentation necessary, the focus is not on the amount of experimentation necessary to practice the entire genus, but the amount of experimentation required to practice any particular member. This is the central holding of *In re Wands*, where the claims read on the use of any IgM antibody that possessed a particular binding affinity. This is similar to the present case where the claims read on increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant which can be readily determined. The *Wands* court recognized that it would require an infinite amount of experimentation to obtain every single possible IgM antibody that could be generated with the specified affinity. Accordingly, the court focused on the amount of experimentation necessary to practice any particular IgM antibody with the recited binding affinity and not the amount of experimentation required to practice the entire genus. This focus is further supported by the multitude of chemical patents that have issued with generic claims reading on tens to hundreds of thousands of individual members. The question then becomes how much experimentation is required to create the claimed invention of increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant. Applicants submit that no more than routine experimentation is required. This may be accomplished by the methods and within the present application and within the technical, scientific skill in the art.

As noted in the specification at page 2, line 22 to page 3, line 3, an important aspect of protein sequence modification is the ability to select a region of the protein

that can be modified without affecting overall structure, stability, function and other cellular and nutritional properties of the protein. Beginning at page 9, line 14, continuing on to page 10, line 12, the specification describes alpha hordothionin modification to increase the amount of lysine, theronine or methionine. As noted there:

> Alpha hordothionin has been modified to increase the amount of various amino acids such as lysine, threonine or methionine. The protein has been synthesized and the three-dimensional structure determined by computer modeling. The modeling of the protein predicts that the ten charged residues (arginine at positions 5, 10, 17, 19 and 30, and lysine at positions 1, 23, 32, 38 and 45) all occur on the surface of the molecule. The side chains of the polar amino acids (asparagine at position 11, glutamine at position 22 and threonine at position 41) also occur on the surface of the molecule. Furthermore, the hydrophobic amino acids, (such as the side chains of leucine at positions 8, 15, 24 and 33 and valine at position 18) are also solvent-accessible.
>
> The Three-dimensional modeling of the protein indicates that the arginine residue at position 10 is important to retention of the appropriate 3-dimensional structure and possible folding through hydrogen bond interactions with the C-terminal residue of the protein. A lysine, methionine or threonine substitution at that point would disrupt this hydrogen bonding network, leading to a destabilization of the structure. The synthetic peptide having this substitution could not be made to fold correctly, which supported this analysis. Conservation of the arginine residue at position 10 provides a protein which folds correctly.
>
> Alpha hordothionin has been modified to contain 12 lysine residues in the mature hordothionin peptide, referred to as HT12. (Rao *et al.* 1994

Protein Engineering 7(12):1485-1493 and WO 94/16078 published July 21, 1994) The disclosure of each of these is incorporated herein by reference in their entirety.

Further analysis of substitutions which would not alter the 3-dimensional structure of the molecule led to replacement of Asparagine-11, Glutamine-22 and Threonine-41 with lysine residues with virtually no steric hindrance. The resulting compound contains 27% lysine residues.

Other combinations of these substitutions were also made, including changes in amino acid residues at one or more of positions 5, 11, 17, 19, 22, 30 and 41 are lysine, and the remainder of the residues at those positions are the residues at the corresponding positions in the wild type hordothionin.

Numerous examples are provided in the specification of proteins appropriate in the invention. Included in such examples is the soybean 2S albumin described in U.S. Patent No. 5,850,016, and the chymotrypsin inhibitor from barley, Williamson et al. *Eur. J. Biochem* 165: 99-108 (1987). Specification page 12, lines 1-5. Production of derivatives of the genes through site directed mutagenesis is also described, and examples provided with the gene encoding barley high lysine polypeptide (BHL), derived from barley chymotrypsin inhibitor, citing PCT/US97/20441. Further, the gene encoding for enhanced soybean albumin gene (ESA) is cited as derived from soybean 2S albumin. Specification page 12, lines 6-14. In addition to an HT12 sequence, an ESA sequence was disclosed as SEQ ID NO: 6, appearing also at Table 2, page 40.

In an amendment of March 11, 2002 (responding to a written description rejection, but which is relevant here as well), in response to a request by the Examiner to point out references showing the availability of such proteins to one skilled in the art, the Applicant provided citations and copies of numerous references. Included among the references were: the 10kD zein storage protein

from maize, Kirihara et al. 1988, *Mol. Gen. Genet.* 211: 477-484; sulfur-rich 10kD rice prolamin, Masumura et al., *Plant Mol. Biol.* 12: 123-130, 1989; pea genes encoding high sulfur protein, Higgins et al., J. *Biol. Chem.*, vol. 261, No. 24, pp. 11124-11131 (1986); 12S seed storage protein gene from rapeseed, Ryan et al., *Nucleic Acids Res.*, 17 (9): 3584 (1989); sunflower 2S albumin gene, Allen et al., *Mol. Gen. Genet.*, 201(2): 211-218 (1987); maize albumin b-32 gene, Di Fonzo et al., *Mol. Gen. Genet.*, 212(3): 481-487 (1988); napin gene, Joseffson et al., *J. Biol. Chem.*, 262 (25): 12202-12208 (1987); B1 hordein gene, Forde et al., *Nucleic Acids Res.* 13(2)): 7327-7339 (1985); wheat alpha and beta gliadin genes, Sumner-Smith et al., *Nucleic Acids Res.*, 13(11): 3905-3916 (1985); wheat gliadin, Anderson et al., *Nucleic Acids Res.*, 12(21): 8129-8144 (1984); pea legumin gene, Lycett et al., *Nucleic Acids Res.*, 12(11): 4493-4506; various maize zeins, disclosed in Heidecker and Messing, *Nucleic Acids Res.*, 11(14): 4891-906 (1983); alpha, alpha', and beta-subunits of soybean 7S seed storage protein, Schuler et al., *Nucleic Acids Res.*, 10(24): 8225-8244 (1982); sunflower 11S gene, Vonder Haar et al., *Gene,* 74(2): 433-443 (1988); and pea convicilin gene, Bown et al., *Biochem. J.*, 251(3): 717-726 (1988). Other references were also provided; these are a sample of the various proteins one skilled has available for use with the invention.

Further, the declaration of Dr. Rudoph Jung of October 18, 1999, shows comparison of control plant lysine, cysteine and methionine content with plants expressing the HT12 sequence and ESA sequence, showing that most of the samples demonstrated at least a 10% increase, or at least a 15% increase, or at least a 20% increase in lysine and in sulfur containing amino acids.

Thus the Applicant submits that one skilled in the art, upon reading the specification, can readily produce an amino acid encoding sequence of the invention. Linking such a protein with an endosperm preferred promoter is likewise well within the abilities of one skilled in the art.

It is the burden of the Patent Office to set forth a reasonable explanation as to why it believes the scope of claim protection is not enabled by the specification. *In*

re Wright, 999 F.2d 1557, 1561-62 (Fed. Cir. 1993). Applicant submits that the Patent Office has not set out a *prima facie* case of non-enablement and any such demonstration has been rebutted by the Applicant.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

Claim 79 is to a transgenic cereal plant seed produced by the method of claim 113 and claim 114 is to a transgenic cereal plant seed comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid. In addition to the enabling disclosures pointed out in regard to claim 113, production of a transgenic plant seed is enabled disclosures which include page 23-27, Example 4 and Table 1, as well as the knowledge of one skilled in the art.

Claim 90

The claim is to an expression cassette according to claim 112 wherein the promoter is a gamma zein promoter or a waxy promoter. In addition to the enabling disclosures directed to claim 112, these are promoters known to one skilled in the art and citations for such well-known promoters are provided at page 18, lines 7-17 in the specification.

Claim 91 and Claim 112

These two claims are to a vector comprising the expression cassette of claim 112 (claim 91) and expression cassette comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine

or about 6 mole % to about 40 mole % of sulfur-containing amino acid (claim 112). In addition to the enabling disclosure and knowledge of one skilled in the art supporting claim 113, the specification provides at Table 2 at page 40 of the specification expression cassettes as examples.

Claim 105

The claim is to a transgenic cereal plant seed of claim 114 where the endosperm-preferred promoter is heterologous to the polynucleotide. In addition to the enabling support for claim 114, one skilled in the art can readily identify an endosperm-preferred promoter heterologous to the polynucleotide, and examples are provided in the specification at page 18, lines 1-18.

Claim 113

This claim is to a method for increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, which method comprises transforming the plant cell with an expression cassette which comprises a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, wherein the level of lysine or sulfur-containing amino acid is increased compared to a non-transgenic plant, and regenerating a plant to produce plant seed. Such a method is taught by the specification, which describes the methodology of producing a polynucleotide encoding such a barley alpha-hordothionine. See the specification at pages 2-3, 9-10 and 12-13, describing methods and alternatives, and Examples 1-4 which provide details for such a method. Specific examples are provided in the specification, and also in the declaration of Dr. Jung. Further, one skilled in the art would appreciate variations in the methodology.

Claim 115 and 116

A transgenic cereal plant (claim 115) or plant cells (claim 116) are claimed comprising an endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid. In addition to the knowledge of one skilled in the art, and the enabling disclosure for claim 113, further description of producing of plants and plant cells is provided in the specification at pages 21, 23-25, Example 4 and Table 1.

VIIIB ARGUMENTS – Rejections Under 35 U.S.C. § 112, Second Paragraph

Rejections of claims 79, 90, 91, 103, 105 and 112-116

Claims 112-116 and dependent claims 79, 90, 91, 103 and 105 are rejected as indefinite and confusing in the recitation of "one or more" with regard to a range of mole percent of lysine and of mole percent of a sulfur containing amino acid, since only two ranges appear. An amendment was submitted January 25, 2005 which amended claims 112-116 to recite "one or both" instead or "one or more." Claim 103 was cancelled in this amendment. Since the amendment complies with the Examiner's request, does not introduce new matter, and merely clarifies confusing language, it is requested that the amendment be entered pursuant to MPEP §1210.

Claims 115 and 116 and all claims dependent thereon are rejected as indefinite in that they are unduly alternative in the multiple recitations of "and/or" in the claims. Applicants point out that claims 115 and 116 were amended in an amendment of January 19, 2004 to remove "and" to recite only "or." Thus it is believed the rejection is moot.

VIIIC ARGUMENTS – REJECTIONS UNDER 35 U.S.C. § 102

Rejection of Claim 103

Claim 103 is rejected under 35 U.S.C. §102(b) as being anticipated by Powell et al , U.S. Patent No. 3,909,288. Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

VIIID ARGUMENTS – REJECTIONS UNDER 35 U.S.C. § 103

Rejection of Claims 79, 90, 91, 103, 105 and 112-116 as being obvious over Rao et al. U.S. Patent 5,885,802 "in view of Applicants' admission"

Claims 79, 90, 91, 103, 105 and 112-116 are rejected over Rao et al., U.S. Patent 5,885,802 "in view of Applicants' admission." The Examiner says that Rao's invention makes the invention obvious in that Rao is to "increasing amino acid composition of the seed (the major portion of which is the endosperm) with the constitutive promoter, and that one would have been motivated to substitute a seed-specific or endosperm-specific promoter to further increase or to limit increases in the seed/endosperm tissue." The admission cited is that endosperm-specific promoters are well known in the art.

Applicant agrees that endosperm-preferred promoters are well known in the art at the time of the invention. The claims are not directed to endosperm-preferred promoters *per se*, but rather to the unexpected result of the use of an endosperm-preferred promoter with a barley alpha-hordothionin protein, modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, in resulting in an increase in the level of lysine or sulfur-containing amino acids when compared to a non-transgenic cereal plant seed. The Rao reference uses a constitutive promoter, and does not teach or

suggest that one can expect improved results when using endosperm-preferred promoters. In fact, the reference teaches away from use of endosperm-preferred promoters, in the statement found at column 5, lines 7-9 that "the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency." There is no motivation to modify the Rao teachings to instead use an endosperm-preferred promoter. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference," *In re Kotzab*, 208 F.3d 1352 (Fed. Cir. 2000).

Such motivation is not found in the teachings, or in the observation that the endosperm is a major portion of the seed of the plant. That, alone, does not motivate one to chose an endosperm-*preferred* promoter to increase lysine and sulfur-containing amino acids in the content of the seed. Why not select a seed-preferred promoter? Then all the tissue will contain the expressed protein. Why not use the strong constitutive promoter of Rao and aim for high expression by use of a strong promoter expressing throughout the plant? No motivation or teaching of success is provided. Applicants submit a *prima facie* case of obviousness has not been presented.

Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

The Rao reference does not teach a transgenic cereal plant seed which has been produced comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain

one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid and increasing lysine or sulfur-containing amino acid compared to a non-transgenic cereal plant.

Claim 90

An expression cassette having a gamma zein or waxy promoter to obtain increased lysine and sulfur-containing amino acids produced from a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid is not taught in Rao.

Claim 91 and Claim 112

Neither a vector comprising the expression cassette of claim 112 (claim 91) nor the expression cassette of claim 112 with an endosperm-preferred promoter are taught by Rao.

Claim 105

The transgenic cereal plant seed of claim 114 having an endosperm-preferred promoter heterologous to the polynucleotide linked to the polynucleotide of the invention is not taught by Rao.

Claim 113

Rao does not teach a method of increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, comprising an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acid levels when compared to a non-transgenic plant.

Claim 115 and 116

The plants and plant cells of Rao do not use, and Rao does not teach an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acids compared to non-transgenic plants.

Rejection of Claims 79, 90, 91, 103, 105 and 112-116 as being obvious over Rao et al. U.S. Patent 5,990,389 "in view of Applicants' admission"

Claims 79, 90, 91, 103, 105 and 112-116 are rejected over Rao et al., U.S. Patent 5,990,389 "in view of Applicants' admission." The Examiner says that Rao's invention makes the invention obvious in that Rao is to "increasing amino acid composition of the seed (the major portion of which is the endosperm) with the constitutive promoter, and that one would have been motivated to substitute a seed-specific or endosperm-specific promoter to further increase or to limit increases in the seed/endosperm tissue." The admission cited is that endosperm-specific promoters are well known in the art.

Applicant agrees that endosperm-preferred promoters are well known in the art at the time of the invention. The claims are not directed to endosperm-preferred promoters *per se*, but rather to the unexpected result of the use of an endosperm-preferred promoter with a barley alpha-hordothionin protein, modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, in resulting in an increase in the level of lysine or sulfur-containing amino acids when compared to a non-transgenic cereal plant seed. The Rao reference uses a constitutive promoter, and does not teach or suggest that one can expect improved results when using endosperm-preferred promoters. In fact, the reference teaches away from use of endosperm-preferred promoters, in the statement found at column 4, lines 65-67 that "the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency." There is no motivation to

modify the Rao teachings to instead use an endosperm-preferred promoter. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference," *In re Kotzab*, 208 F.3d 1352 (Fed. Cir. 2000).

Such motivation is not found in the teachings, or in the observation that the endosperm is a major portion of the seed of the plant. That, alone, does not motivate one to choose an endosperm-*preferred* promoter to increase lysine and sulfur-containing amino acids in the content of the seed. Why not select a seed-preferred promoter? Then all the tissue will contain the expressed protein. Why not use the strong constitutive promoter of Rao and aim for high expression by use of a strong promoter expressing throughout the plant? No motivation or teaching of success is provided. Applicants submit a *prima facie* case of obviousness has not been presented.

Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

The Rao reference does not teach a transgenic cereal plant seed which has been produced comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid and increasing lysine or sulfur-containing amino acid compared to a non-transgenic cereal plant.

Claim 90

An expression cassette having a gamma zein or waxy promoter to obtain increased lysine and sulfur-containing amino acids produced from a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid is not taught in Rao.

Claim 91 and Claim 112

Neither a vector comprising the expression cassette of claim 112 (claim 91) nor the expression cassette of claim 112 with an endosperm-preferred promoter are taught by Rao.

Claim 105

The transgenic cereal plant seed of claim 114 having an endosperm-preferred promoter heterologous to the polynucleotide linked to the polynucleotide of the invention is not taught by Rao.

Claim 113

Rao does not teach a method of increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, comprising an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acid levels when compared to a non-transgenic plant.

Claim 115 and 116

The plants and plant cells of Rao do not use, and Rao does not teach an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acids compared to non-transgenic plants.

IX APPENDIX OF CLAIMS

The text of the claims involved in the appeal are:

79. A transgenic cereal plant seed produced by the method of claim 113.

90. The expression cassette according to claim 112 wherein the promoter is a gamma zein promoter or a waxy promoter.

91. A vector comprising the expression cassette of claim 112.

105. The transgenic cereal plant seed of claim 114 wherein the seed endosperm-preferred promoter is heterologous to the polynucleotide.

112. An expression cassette comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid.

113. A method for increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant seed, the method comprising:

a) transforming a cereal plant cell with an expression cassette, and

b) regenerating a transgenic cereal plant to produce a transgenic cereal plant seed,

wherein the expression cassette comprises a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein the level of lysine or sulfur-containing amino acid is increased in the transgenic cereal plant seed compared to a corresponding non-transgenic cereal plant seed.

114. A transgenic cereal plant seed comprising a modified barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein the transgenic cereal plant seed comprises an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

115. A transgenic cereal plant comprising a modified barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein

modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein transgenic seed of the transgenic cereal plant comprise an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

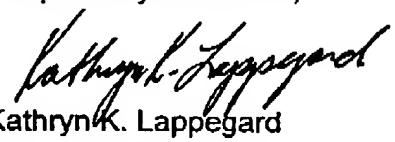
116. A transgenic cereal plant cell comprising a barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein transgenic seed resulting from the transgenic plant cell comprise one or both of an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

Serial No. 09/020,716
Appellant's Brief Dated 03/17/2005

SUMMARY

In view of the above comments, overruling of the outstanding rejections and allowance of all the remaining claims is respectfully requested.

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Jung et al.
Application No.	:	09/020,716
Filed	:	02/09/1998
Group Art Unit	:	1638
Examiner	:	McElwain, Elizabeth F.
For	:	Alteration of Amino Acid Compositions in Seeds

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S BRIEF (37 C.F.R. § 1.192)

This brief is in furtherance of the Notice of Appeal, filed in this case on January 25, 2005.

The fees required under § 1.17, and any required petition for extension of time for filing this brief and fees therefor, are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief is transmitted in triplicate. (37 C.F.R. § 1.192(a).

This brief contains these items under the following headings, and in the order set forth below (37 C.F.R. § 1.192(c)).

I REAL PARTY OF INTEREST
II RELATED APPEALS AND INTERFERENCES
III STATUS OF CLAIMS
IV STATUS OF AMENDMENTS
V SUMMARY OF INVENTION

VI ISSUES

VII GROUPING OF CLAIMS

VIII ARGUMENTS

(check each category of ARGUMENT submitted in this brief)

☒ ARGUMENT: VIIIA REJECTIONS UNDER 35 USC 112, FIRST PARAGRAPH

☒ ARGUMENT: VIIIB REJECTIONS UNDER 35 USC 112, SECOND PARAGRAPH

☒ ARGUMENT: VIIIC REJECTIONS UNDER 35 USC 102

☒ ARGUMENT: VIIID REJECTIONS UNDER 35 USC 103

☐ ARGUMENT: VIIIE REJECTIONS OTHER THAN 35 USC 102, 103, AND 112

IX APPENDIX OF CLAIMS INVOLVED IN THE APPEAL

☐ OTHER MATERIALS THAT APPELLANT CONSIDERS NECESSARY OR DESIRABLE

The final page of this brief bears the practitioner's signature.

I REAL PARTY IN INTEREST

The subject application is owned by Pioneer Hi-Bred International, Inc. of Johnston, Iowa.

II RELATED APPEALS AND INTERFERENCES

With respect to other appeals or interferences that will directly affect, or be directly affected by, or have a bearing on the Board's decision in this appeal, there are no such appeals or interferences.

III STATUS OF CLAIMS

The status of the claims in this application are:

A. TOTAL NUMBER OF CLAIMS IN APPLICATION

Claims in the application are: 1-120

B. STATUS OF ALL THE CLAIMS

1. Claims cancelled: 1-78, 80-89, 92-104, 106-111, and 117-120
2. Claims withdrawn from consideration but not cancelled: None
3. Claims pending: 79, 90, 91, 105, and 112-116
4. Claims allowed: None
5. Claims rejected: 79, 90, 91, 105, 112-116

C. CLAIMS ON APPEAL

The claims on appeal are: 79, 90, 91, 105, and 112-116

IV STATUS OF AMENDMENTS

Insofar as understood by the appellant, the status of the amendment filed January 25, 2005, has not been acted upon by the Examiner nor entered.

V SUMMARY OF INVENTION

The present invention provides methods for increasing the level of lysine, sulfur-containing amino acids or both, in a cereal plant. Specification page 4, lines 5-19. The invention employs a promoter with endosperm-preferred expression. Specification page 4, line 20 to specification, page 5, line 7. The promoter is linked to a barley alpha-hordothionin polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid. Specification page 6, lines 21 to page 7 line 4 and specification page 8, line 3 to page 10, line 20. Also provided are expression cassettes and plant seed produced by such method. Specification page 4, lines 5-6 and page 5, lines 1-12.

Crop plants in feed formulations must typically be supplemented with specific amino acids in order to provide animals consuming the plants with essential nutrients necessary for their growth. Lysine, methionine and threonine are among the amino acids necessary for animal nutrition. Various attempts have been made to increase the level of these amino acids, which attempts have been met with limited success. See the specification, page 1.

One means of enhancing levels of amino acid levels of crop plants is modification of amino acid biosynthesis in the plant. In the approach of protein sequence modification, a gene is identified encoding a major protein as the target for modification to contain more codons of essential amino acids. Specification, page 2, lines 1-2 and lines 22-23. An important approach is to select a region of the protein that can be modified without affecting the overall structure, stability, function and other cellular and nutritional properties of the protein. Specification page 2, line 22 to page 3, line 3. In one example of attempts to achieve this goal to date, expression of the *lysC* gene resulted in increase methionine and threonine biosynthesis, but only a 6-7% increase in total threonine or methionine in seed. Specification, page 2, lines 10-19. Use of sulfur-rich Brazil Nut Protein increased methionine content, but decreased lysine content and further has been identified as a major food allergen. Specification, page 3, lines 14-23. Therefore there is a need to improve the nutritional value of plant seeds without accompanying detrimental side affects. Specification page 4, lines 1-3.

VI ISSUES

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

Claim 103 is rejected under 35 U.S.C. §102(b) as being anticipated by Powell et al, U.S. Patent No. 3,909,288.

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §103 as being obvious over Rao et al., U.S. patent 5,885,802 "in view of Applicants' admission."

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §103 as being obvious over Rao et al., U.S. patent 5,990,389 "in view of Applicants' admission."

VII GROUPING OF CLAIMS

The claims do not stand or fall together except as regards the second paragraph section 112 second paragraph rejection. The patentability of the claims will be argued separately.

VIIIA ARGUMENTS – Rejections Under 35 U.S.C. § 112, First Paragraph

Rejection of Claims 79, 90, 91, 103, 105 and 112-116

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. The Examiner states the specification teaches a transformed cereal plant seed having an elevated lysine, methionine and cysteine content (about 10% to about 35% by weight compared to untransformed cereal plant seed) comprising the modified hordothionin gene of SEQ ID NO: 2 (HT-12), vectors, plant cells and transformed plants comprising said modified hordothionin gene. The Examiner finds the specification does not teach the range of 7 mole% to 50 mole% of lysine or 6 mole % to 40 mole% of sulfur-containing amino acids for any seed protein, and further does not teach any seed proteins having both of these ranges. The Examiner says that working examples need to be provided, and there is an absence of guidance in identifying such sequences and transforming into plants.

In response to the rejection, the Applicant pointed out that the specification includes a number of examples of proteins useful in the invention and teaches one how to identify such proteins. The Examiner says that the example of a construct of HT-12 and an endosperm preferred promoter is not adequate. A declaration was provided by Dr. Rudolph Jung demonstrating production of proteins of the invention using a construct with sequences encoding modified hordothionin HT-12 as well as soybean 2S albumin (ESA) as compared with a control. The Examiner responded that one working example is not sufficient and "the example provided in the Declaration of Jung does not provide information to support the claims."

Claim 103 was cancelled in the amendment after of January 25, 2005. Since the amendment complies with the Examiner's request, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

The claims recite a method, cereal plant seed, or cassette comprising a modified barley alpha-hordothionin polynucleotide, operably linked to a seed endosperm-preferred promoter, which nucleotide encodes a barley alpha-hordothionin protein modified to contain about 7 mole %to about 40 mole % lysine or 6 mole % to about 40 mole % of a sulfur-containing amino acid, resulting in increased levels of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

The Examiner's primary issue, as outlined in the last Office Action, appears to be directed to the "lack of working examples of protein coding sequences having the claimed ranges of mole % lysine and sulfur containing amino acids" and identifying and transforming such sequences. The Examiner focuses on the "one" example. However, the Applicant has demonstrated that there are various methods to identify proteins to be used in the invention, listed various sequences encoding proteins useful in the invention, and described methodology by which to identify the proteins. Compliance with the enablement requirement of section 112 "does not turn on whether an example is disclosed. All that is required by the law is an objective enablement of the invention, whether by working examples or by the use of broad

terminology." *In re Vaeck*, 947 F2d 488, 496 (Fed. Cir. 1991). Eight considerations are indicated in *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988) in determining if undue experimentation is necessary to practice an invention. These are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence of working examples of the invention, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art; and the breadth of the claims.

When determining the quantity of experimentation necessary, the focus is not on the amount of experimentation necessary to practice the entire genus, but the amount of experimentation required to practice any particular member. This is the central holding of *In re Wands,* where the claims read on the use of any IgM antibody that possessed a particular binding affinity. This is similar to the present case where the claims read on increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant which can be readily determined. The *Wands* court recognized that it would require an infinite amount of experimentation to obtain every single possible IgM antibody that could be generated with the specified affinity. Accordingly, the court focused on the amount of experimentation necessary to practice any particular IgM antibody with the recited binding affinity and not the amount of experimentation required to practice the entire genus. This focus is further supported by the multitude of chemical patents that have issued with generic claims reading on tens to hundreds of thousands of individual members. The question then becomes how much experimentation is required to create the claimed invention of increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant. Applicants submit that no more than routine experimentation is required. This may be accomplished by the methods and within the present application and within the technical, scientific skill in the art.

As noted in the specification at page 2, line 22 to page 3, line 3, an important aspect of protein sequence modification is the ability to select a region of the protein

that can be modified without affecting overall structure, stability, function and other cellular and nutritional properties of the protein. Beginning at page 9, line 14, continuing on to page 10, line 12, the specification describes alpha hordothionin modification to increase the amount of lysine, theronine or methionine. As noted there:

> Alpha hordothionin has been modified to increase the amount of various amino acids such as lysine, threonine or methionine. The protein has been synthesized and the three-dimensional structure determined by computer modeling. The modeling of the protein predicts that the ten charged residues (arginine at positions 5, 10, 17, 19 and 30, and lysine at positions 1, 23, 32, 38 and 45) all occur on the surface of the molecule. The side chains of the polar amino acids (asparagine at position 11, glutamine at position 22 and threonine at position 41) also occur on the surface of the molecule. Furthermore, the hydrophobic amino acids, (such as the side chains of leucine at positions 8, 15, 24 and 33 and valine at position 18) are also solvent-accessible.
>
> The Three-dimensional modeling of the protein indicates that the arginine residue at position 10 is important to retention of the appropriate 3-dimensional structure and possible folding through hydrogen bond interactions with the C-terminal residue of the protein. A lysine, methionine or threonine substitution at that point would disrupt this hydrogen bonding network, leading to a destabilization of the structure. The synthetic peptide having this substitution could not be made to fold correctly, which supported this analysis. Conservation of the arginine residue at position 10 provides a protein which folds correctly.
>
> Alpha hordothionin has been modified to contain 12 lysine residues in the mature hordothionin peptide, referred to as HT12. (Rao *et al.* 1994

Protein Engineering 7(12):1485-1493 and WO 94/16078 published July 21, 1994) The disclosure of each of these is incorporated herein by reference in their entirety.

Further analysis of substitutions which would not alter the 3-dimensional structure of the molecule led to replacement of Asparagine-11, Glutamine-22 and Threonine-41 with lysine residues with virtually no steric hindrance. The resulting compound contains 27% lysine residues.

Other combinations of these substitutions were also made, including changes in amino acid residues at one or more of positions 5, 11, 17, 19, 22, 30 and 41 are lysine, and the remainder of the residues at those positions are the residues at the corresponding positions in the wild type hordothionin.

Numerous examples are provided in the specification of proteins appropriate in the invention. Included in such examples is the soybean 2S albumin described in U.S. Patent No. 5,850,016, and the chymotrypsin inhibitor from barley, Williamson et al. *Eur. J. Biochem* 165: 99-108 (1987). Specification page 12, lines 1-5. Production of derivatives of the genes through site directed mutagenesis is also described, and examples provided with the gene encoding barley high lysine polypeptide (BHL), derived from barley chymotrypsin inhibitor, citing PCT/US97/20441. Further, the gene encoding for enhanced soybean albumin gene (ESA) is cited as derived from soybean 2S albumin. Specification page 12, lines 6-14. In addition to an HT12 sequence, an ESA sequence was disclosed as SEQ ID NO: 6, appearing also at Table 2, page 40.

In an amendment of March 11, 2002 (responding to a written description rejection, but which is relevant here as well), in response to a request by the Examiner to point out references showing the availability of such proteins to one skilled in the art, the Applicant provided citations and copies of numerous references. Included among the references were: the 10kD zein storage protein

from maize, Kirihara et al. 1988, *Mol. Gen. Genet.* 211: 477-484; sulfur-rich 10kD rice prolamin, Masumura et al., *Plant Mol. Biol.* 12: 123-130, 1989; pea genes encoding high sulfur protein, Higgins et al., *J. Biol. Chem.*, vol. 261, No. 24, pp. 11124-11131 (1986); 12S seed storage protein gene from rapeseed, Ryan et al., *Nucleic Acids Res.*, 17 (9): 3584 (1989); sunflower 2S albumin gene, Allen et al., *Mol. Gen. Genet.*, 201(2): 211-218 (1987); maize albumin b-32 gene, Di Fonzo et al., *Mol. Gen. Genet.*, 212(3): 481-487 (1988); napin gene, Joseffson et al., *J. Biol. Chem.*, 262 (25): 12202-12208 (1987); B1 hordein gene, Forde et al., *Nucleic Acids Res.* 13(2)): 7327-7339 (1985); wheat alpha and beta gliadin genes, Sumner-Smith et al., *Nucleic Acids Res.*, 13(11): 3905-3916 (1985); wheat gliadin, Anderson et al., *Nucleic Acids Res.*, 12(21): 8129-8144 (1984); pea legumin gene, Lycett et al., *Nucleic Acids Res.*, 12(11): 4493-4506; various maize zeins, disclosed in Heidecker and Messing, *Nucleic Acids Res.*, 11(14): 4891-906 (1983); alpha, alpha', and beta-subunits of soybean 7S seed storage protein, Schuler et al., *Nucleic Acids Res.*, 10(24): 8225-8244 (1982); sunflower 11S gene, Vonder Haar et al., *Gene*, 74(2): 433-443 (1988); and pea convicilin gene, Bown et al., *Biochem. J.*, 251(3): 717-726 (1988). Other references were also provided; these are a sample of the various proteins one skilled has available for use with the invention.

Further, the declaration of Dr. Rudoph Jung of October 18, 1999, shows comparison of control plant lysine, cysteine and methionine content with plants expressing the HT12 sequence and ESA sequence, showing that most of the samples demonstrated at least a 10% increase, or at least a 15% increase, or at least a 20% increase in lysine and in sulfur containing amino acids.

Thus the Applicant submits that one skilled in the art, upon reading the specification, can readily produce an amino acid encoding sequence of the invention. Linking such a protein with an endosperm preferred promoter is likewise well within the abilities of one skilled in the art.

It is the burden of the Patent Office to set forth a reasonable explanation as to why it believes the scope of claim protection is not enabled by the specification. *In*

re Wright, 999 F.2d 1557, 1561-62 (Fed. Cir. 1993). Applicant submits that the Patent Office has not set out a *prima facie* case of non-enablement and any such demonstration has been rebutted by the Applicant.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

Claim 79 is to a transgenic cereal plant seed produced by the method of claim 113 and claim 114 is to a transgenic cereal plant seed comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid. In addition to the enabling disclosures pointed out in regard to claim 113, production of a transgenic plant seed is enabled disclosures which include page 23-27, Example 4 and Table 1, as well as the knowledge of one skilled in the art.

Claim 90

The claim is to an expression cassette according to claim 112 wherein the promoter is a gamma zein promoter or a waxy promoter. In addition to the enabling disclosures directed to claim 112, these are promoters known to one skilled in the art and citations for such well-known promoters are provided at page 18, lines 7-17 in the specification.

Claim 91 and Claim 112

These two claims are to a vector comprising the expression cassette of claim 112 (claim 91) and expression cassette comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine

or about 6 mole % to about 40 mole % of sulfur-containing amino acid (claim 112). In addition to the enabling disclosure and knowledge of one skilled in the art supporting claim 113, the specification provides at Table 2 at page 40 of the specification expression cassettes as examples.

Claim 105

The claim is to a transgenic cereal plant seed of claim 114 where the endosperm-preferred promoter is heterologous to the polynucleotide. In addition to the enabling support for claim 114, one skilled in the art can readily identify an endosperm-preferred promoter heterologous to the polynucleotide, and examples are provided in the specification at page 18, lines 1-18.

Claim 113

This claim is to a method for increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, which method comprises transforming the plant cell with an expression cassette which comprises a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, wherein the level of lysine or sulfur-containing amino acid is increased compared to a non-transgenic plant, and regenerating a plant to produce plant seed. Such a method is taught by the specification, which describes the methodology of producing a polynucleotide encoding such a barley alpha-hordothionine. See the specification at pages 2-3, 9-10 and 12-13, describing methods and alternatives, and Examples 1-4 which provide details for such a method. Specific examples are provided in the specification, and also in the declaration of Dr. Jung. Further, one skilled in the art would appreciate variations in the methodology.

Claim 115 and 116

A transgenic cereal plant (claim 115) or plant cells (claim 116) are claimed comprising an endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid. In addition to the knowledge of one skilled in the art, and the enabling disclosure for claim 113, further description of producing of plants and plant cells is provided in the specification at pages 21, 23-25, Example 4 and Table 1.

VIIIB ARGUMENTS – Rejections Under 35 U.S.C. § 112, Second Paragraph

Rejections of claims 79, 90, 91, 103, 105 and 112-116

Claims 112-116 and dependent claims 79, 90, 91, 103 and 105 are rejected as indefinite and confusing in the recitation of "one or more" with regard to a range of mole percent of lysine and of mole percent of a sulfur containing amino acid, since only two ranges appear. An amendment was submitted January 25, 2005 which amended claims 112-116 to recite "one or both" instead or "one or more." Claim 103 was cancelled in this amendment. Since the amendment complies with the Examiner's request, does not introduce new matter, and merely clarifies confusing language, it is requested that the amendment be entered pursuant to MPEP §1210.

Claims 115 and 116 and all claims dependent thereon are rejected as indefinite in that they are unduly alternative in the multiple recitations of "and/or" in the claims. Applicants point out that claims 115 and 116 were amended in an amendment of January 19, 2004 to remove "and" to recite only "or." Thus it is believed the rejection is moot.

VIIIC ARGUMENTS – REJECTIONS UNDER 35 U.S.C. § 102

Rejection of Claim 103

Claim 103 is rejected under 35 U.S.C. §102(b) as being anticipated by Powell et al , U.S. Patent No. 3,909,288. Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

VIIID ARGUMENTS – REJECTIONS UNDER 35 U.S.C. § 103

Rejection of Claims 79, 90, 91, 103, 105 and 112-116 as being obvious over Rao et al. U.S. Patent 5,885,802 "in view of Applicants' admission"

Claims 79, 90, 91, 103, 105 and 112-116 are rejected over Rao et al., U.S. Patent 5,885,802 "in view of Applicants' admission." The Examiner says that Rao's invention makes the invention obvious in that Rao is to "increasing amino acid composition of the seed (the major portion of which is the endosperm) with the constitutive promoter, and that one would have been motivated to substitute a seed-specific or endosperm-specific promoter to further increase or to limit increases in the seed/endosperm tissue." The admission cited is that endosperm-specific promoters are well known in the art.

Applicant agrees that endosperm-preferred promoters are well known in the art at the time of the invention. The claims are not directed to endosperm-preferred promoters *per se*, but rather to the unexpected result of the use of an endosperm-preferred promoter with a barley alpha-hordothionin protein, modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, in resulting in an increase in the level of lysine or sulfur-containing amino acids when compared to a non-transgenic cereal plant seed. The Rao reference uses a constitutive promoter, and does not teach or

suggest that one can expect improved results when using endosperm-preferred promoters. In fact, the reference teaches away from use of endosperm-preferred promoters, in the statement found at column 5, lines 7-9 that "the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency." There is no motivation to modify the Rao teachings to instead use an endosperm-preferred promoter. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference," *In re Kotzab*, 208 F.3d 1352 (Fed. Cir. 2000).

Such motivation is not found in the teachings, or in the observation that the endosperm is a major portion of the seed of the plant. That, alone, does not motivate one to chose an endosperm-*preferred* promoter to increase lysine and sulfur-containing amino acids in the content of the seed. Why not select a seed-preferred promoter? Then all the tissue will contain the expressed protein. Why not use the strong constitutive promoter of Rao and aim for high expression by use of a strong promoter expressing throughout the plant? No motivation or teaching of success is provided. Applicants submit a *prima facie* case of obviousness has not been presented.

Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

The Rao reference does not teach a transgenic cereal plant seed which has been produced comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain

one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid and increasing lysine or sulfur-containing amino acid compared to a non-transgenic cereal plant.

Claim 90

An expression cassette having a gamma zein or waxy promoter to obtain increased lysine and sulfur-containing amino acids produced from a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid is not taught in Rao.

Claim 91 and Claim 112

Neither a vector comprising the expression cassette of claim 112 (claim 91) nor the expression cassette of claim 112 with an endosperm-preferred promoter are taught by Rao.

Claim 105

The transgenic cereal plant seed of claim 114 having an endosperm-preferred promoter heterologous to the polynucleotide linked to the polynucleotide of the invention is not taught by Rao.

Claim 113

Rao does not teach a method of increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, comprising an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acid levels when compared to a non-transgenic plant.

Claim 115 and 116

The plants and plant cells of Rao do not use, and Rao does not teach an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acids compared to non-transgenic plants.

Rejection of Claims 79, 90, 91, 103, 105 and 112-116 as being obvious over Rao et al. U.S. Patent 5,990,389 "in view of Applicants' admission"

Claims 79, 90, 91, 103, 105 and 112-116 are rejected over Rao et al., U.S. Patent 5,990,389 "in view of Applicants' admission." The Examiner says that Rao's invention makes the invention obvious in that Rao is to "increasing amino acid composition of the seed (the major portion of which is the endosperm) with the constitutive promoter, and that one would have been motivated to substitute a seed-specific or endosperm-specific promoter to further increase or to limit increases in the seed/endosperm tissue." The admission cited is that endosperm-specific promoters are well known in the art.

Applicant agrees that endosperm-preferred promoters are well known in the art at the time of the invention. The claims are not directed to endosperm-preferred promoters *per se*, but rather to the unexpected result of the use of an endosperm-preferred promoter with a barley alpha-hordothionin protein, modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, in resulting in an increase in the level of lysine or sulfur-containing amino acids when compared to a non-transgenic cereal plant seed. The Rao reference uses a constitutive promoter, and does not teach or suggest that one can expect improved results when using endosperm-preferred promoters. In fact, the reference teaches away from use of endosperm-preferred promoters, in the statement found at column 4, lines 65-67 that "the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency." There is no motivation to

modify the Rao teachings to instead use an endosperm-preferred promoter. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference," *In re Kotzab*, 208 F.3d 1352 (Fed. Cir. 2000).

Such motivation is not found in the teachings, or in the observation that the endosperm is a major portion of the seed of the plant. That, alone, does not motivate one to choose an endosperm-*preferred* promoter to increase lysine and sulfur-containing amino acids in the content of the seed. Why not select a seed-preferred promoter? Then all the tissue will contain the expressed protein. Why not use the strong constitutive promoter of Rao and aim for high expression by use of a strong promoter expressing throughout the plant? No motivation or teaching of success is provided. Applicants submit a *prima facie* case of obviousness has not been presented.

Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

The Rao reference does not teach a transgenic cereal plant seed which has been produced comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid and increasing lysine or sulfur-containing amino acid compared to a non-transgenic cereal plant.

Claim 90

An expression cassette having a gamma zein or waxy promoter to obtain increased lysine and sulfur-containing amino acids produced from a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid is not taught in Rao.

Claim 91 and Claim 112

Neither a vector comprising the expression cassette of claim 112 (claim 91) nor the expression cassette of claim 112 with an endosperm-preferred promoter are taught by Rao.

Claim 105

The transgenic cereal plant seed of claim 114 having an endosperm-preferred promoter heterologous to the polynucleotide linked to the polynucleotide of the invention is not taught by Rao.

Claim 113

Rao does not teach a method of increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, comprising an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acid levels when compared to a non-transgenic plant.

Claim 115 and 116

The plants and plant cells of Rao do not use, and Rao does not teach an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acids compared to non-transgenic plants.

IX APPENDIX OF CLAIMS

The text of the claims involved in the appeal are:

79. A transgenic cereal plant seed produced by the method of claim 113.

90. The expression cassette according to claim 112 wherein the promoter is a gamma zein promoter or a waxy promoter.

91. A vector comprising the expression cassette of claim 112.

105. The transgenic cereal plant seed of claim 114 wherein the seed endosperm-preferred promoter is heterologous to the polynucleotide.

112. An expression cassette comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid.

113. A method for increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant seed, the method comprising:

a) transforming a cereal plant cell with an expression cassette, and

b) regenerating a transgenic cereal plant to produce a transgenic cereal plant seed,

wherein the expression cassette comprises a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein the level of lysine or sulfur-containing amino acid is increased in the transgenic cereal plant seed compared to a corresponding non-transgenic cereal plant seed.

114. A transgenic cereal plant seed comprising a modified barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein the transgenic cereal plant seed comprises an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

115. A transgenic cereal plant comprising a modified barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein

modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein transgenic seed of the transgenic cereal plant comprise an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

116. A transgenic cereal plant cell comprising a barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein transgenic seed resulting from the transgenic plant cell comprise one or both of an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

SUMMARY

In view of the above comments, overruling of the outstanding rejections and allowance of all the remaining claims is respectfully requested.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Jung et al.
Application No.	:	09/020,716
Filed	:	02/09/1998
Group Art Unit	:	1638
Examiner	:	McElwain, Elizabeth F.
For	:	Alteration of Amino Acid Compositions in Seeds

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S BRIEF (37 C.F.R. § 1.192)

This brief is in furtherance of the Notice of Appeal, filed in this case on January 25, 2005.

The fees required under § 1.17, and any required petition for extension of time for filing this brief and fees therefor, are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief is transmitted in triplicate. (37 C.F.R. § 1.192(a).

This brief contains these items under the following headings, and in the order set forth below (37 C.F.R. § 1.192(c)).

I REAL PARTY OF INTEREST
II RELATED APPEALS AND INTERFERENCES
III STATUS OF CLAIMS
IV STATUS OF AMENDMENTS
V SUMMARY OF INVENTION

VI ISSUES

VII GROUPING OF CLAIMS

VIII ARGUMENTS

(check each category of ARGUMENT submitted in this brief)

- ☒ ARGUMENT: VIIIA REJECTIONS UNDER 35 USC 112, FIRST PARAGRAPH
- ☒ ARGUMENT: VIIIB REJECTIONS UNDER 35 USC 112, SECOND PARAGRAPH
- ☒ ARGUMENT: VIIIC REJECTIONS UNDER 35 USC 102
- ☒ ARGUMENT: VIIID REJECTIONS UNDER 35 USC 103
- ☐ ARGUMENT: VIIIE REJECTIONS OTHER THAN 35 USC 102, 103, AND 112

IX APPENDIX OF CLAIMS INVOLVED IN THE APPEAL

☐ OTHER MATERIALS THAT APPELLANT CONSIDERS NECESSARY OR DESIRABLE

The final page of this brief bears the practitioner's signature.

I REAL PARTY IN INTEREST

The subject application is owned by Pioneer Hi-Bred International, Inc. of Johnston, Iowa.

II RELATED APPEALS AND INTERFERENCES

With respect to other appeals or interferences that will directly affect, or be directly affected by, or have a bearing on the Board's decision in this appeal, there are no such appeals or interferences.

III STATUS OF CLAIMS

The status of the claims in this application are:

A. TOTAL NUMBER OF CLAIMS IN APPLICATION

Claims in the application are: 1-120

B. STATUS OF ALL THE CLAIMS

1. Claims cancelled: 1-78, 80-89, 92-104, 106-111, and 117-120
2. Claims withdrawn from consideration but not cancelled: None
3. Claims pending: 79, 90, 91, 105, and 112-116
4. Claims allowed: None
5. Claims rejected: 79, 90, 91, 105, 112-116

C. CLAIMS ON APPEAL

The claims on appeal are: 79, 90, 91, 105, and 112-116

IV STATUS OF AMENDMENTS

Insofar as understood by the appellant, the status of the amendment filed January 25, 2005, has not been acted upon by the Examiner nor entered.

V SUMMARY OF INVENTION

The present invention provides methods for increasing the level of lysine, sulfur-containing amino acids or both, in a cereal plant. Specification page 4, lines 5-19. The invention employs a promoter with endosperm-preferred expression. Specification page 4, line 20 to specification, page 5, line 7. The promoter is linked to a barley alpha-hordothionin polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid. Specification page 6, lines 21 to page 7 line 4 and specification page 8, line 3 to page 10, line 20. Also provided are expression cassettes and plant seed produced by such method. Specification page 4, lines 5-6 and page 5, lines 1-12.

Crop plants in feed formulations must typically be supplemented with specific amino acids in order to provide animals consuming the plants with essential nutrients necessary for their growth. Lysine, methionine and threonine are among the amino acids necessary for animal nutrition. Various attempts have been made to increase the level of these amino acids, which attempts have been met with limited success. See the specification, page 1.

One means of enhancing levels of amino acid levels of crop plants is modification of amino acid biosynthesis in the plant. In the approach of protein sequence modification, a gene is identified encoding a major protein as the target for modification to contain more codons of essential amino acids. Specification, page 2, lines 1-2 and lines 22-23. An important approach is to select a region of the protein that can be modified without affecting the overall structure, stability, function and other cellular and nutritional properties of the protein. Specification page 2, line 22 to page 3, line 3. In one example of attempts to achieve this goal to date, expression of the *lysC* gene resulted in increase methionine and threonine biosynthesis, but only a 6-7% increase in total threonine or methionine in seed. Specification, page 2, lines 10-19. Use of sulfur-rich Brazil Nut Protein increased methionine content, but decreased lysine content and further has been identified as a major food allergen. Specification, page 3, lines 14-23. Therefore there is a need to improve the nutritional value of plant seeds without accompanying detrimental side affects. Specification page 4, lines 1-3.

VI ISSUES

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

Claim 103 is rejected under 35 U.S.C. §102(b) as being anticipated by Powell et al, U.S. Patent No. 3,909,288.

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §103 as being obvious over Rao et al., U.S. patent 5,885,802 "in view of Applicants' admission."

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. §103 as being obvious over Rao et al., U.S. patent 5,990,389 "in view of Applicants' admission."

VII GROUPING OF CLAIMS

The claims do not stand or fall together except as regards the second paragraph section 112 second paragraph rejection. The patentability of the claims will be argued separately.

VIIIA ARGUMENTS – Rejections Under 35 U.S.C. § 112, First Paragraph

Rejection of Claims 79, 90, 91, 103, 105 and 112-116

Claims 79, 90, 91, 103, 105 and 112-116 are rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. The Examiner states the specification teaches a transformed cereal plant seed having an elevated lysine, methionine and cysteine content (about 10% to about 35% by weight compared to untransformed cereal plant seed) comprising the modified hordothionin gene of SEQ ID NO: 2 (HT-12), vectors, plant cells and transformed plants comprising said modified hordothionin gene. The Examiner finds the specification does not teach the range of 7 mole% to 50 mole% of lysine or 6 mole % to 40 mole% of sulfur-containing amino acids for any seed protein, and further does not teach any seed proteins having both of these ranges. The Examiner says that working examples need to be provided, and there is an absence of guidance in identifying such sequences and transforming into plants.

In response to the rejection, the Applicant pointed out that the specification includes a number of examples of proteins useful in the invention and teaches one how to identify such proteins. The Examiner says that the example of a construct of HT-12 and an endosperm preferred promoter is not adequate. A declaration was provided by Dr. Rudolph Jung demonstrating production of proteins of the invention using a construct with sequences encoding modified hordothionin HT-12 as well as soybean 2S albumin (ESA) as compared with a control. The Examiner responded that one working example is not sufficient and "the example provided in the Declaration of Jung does not provide information to support the claims."

Claim 103 was cancelled in the amendment after of January 25, 2005. Since the amendment complies with the Examiner's request, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

The claims recite a method, cereal plant seed, or cassette comprising a modified barley alpha-hordothionin polynucleotide, operably linked to a seed endosperm-preferred promoter, which nucleotide encodes a barley alpha-hordothionin protein modified to contain about 7 mole %to about 40 mole % lysine or 6 mole % to about 40 mole % of a sulfur-containing amino acid, resulting in increased levels of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

The Examiner's primary issue, as outlined in the last Office Action, appears to be directed to the "lack of working examples of protein coding sequences having the claimed ranges of mole % lysine and sulfur containing amino acids" and identifying and transforming such sequences. The Examiner focuses on the "one" example. However, the Applicant has demonstrated that there are various methods to identify proteins to be used in the invention, listed various sequences encoding proteins useful in the invention, and described methodology by which to identify the proteins. Compliance with the enablement requirement of section 112 "does not turn on whether an example is disclosed. All that is required by the law is an objective enablement of the invention, whether by working examples or by the use of broad

terminology." *In re Vaeck*, 947 F2d 488, 496 (Fed. Cir. 1991). Eight considerations are indicated in *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988) in determining if undue experimentation is necessary to practice an invention. These are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence of working examples of the invention, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims.

When determining the quantity of experimentation necessary, the focus is not on the amount of experimentation necessary to practice the entire genus, but the amount of experimentation required to practice any particular member. This is the central holding of *In re Wands*, where the claims read on the use of any IgM antibody that possessed a particular binding affinity. This is similar to the present case where the claims read on increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant which can be readily determined. The *Wands* court recognized that it would require an infinite amount of experimentation to obtain every single possible IgM antibody that could be generated with the specified affinity. Accordingly, the court focused on the amount of experimentation necessary to practice any particular IgM antibody with the recited binding affinity and not the amount of experimentation required to practice the entire genus. This focus is further supported by the multitude of chemical patents that have issued with generic claims reading on tens to hundreds of thousands of individual members. The question then becomes how much experimentation is required to create the claimed invention of increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant. Applicants submit that no more than routine experimentation is required. This may be accomplished by the methods and within the present application and within the technical, scientific skill in the art.

As noted in the specification at page 2, line 22 to page 3, line 3, an important aspect of protein sequence modification is the ability to select a region of the protein

that can be modified without affecting overall structure, stability, function and other cellular and nutritional properties of the protein. Beginning at page 9, line 14, continuing on to page 10, line 12, the specification describes alpha hordothionin modification to increase the amount of lysine, theronine or methionine. As noted there:

> Alpha hordothionin has been modified to increase the amount of various amino acids such as lysine, threonine or methionine. The protein has been synthesized and the three-dimensional structure determined by computer modeling. The modeling of the protein predicts that the ten charged residues (arginine at positions 5, 10, 17, 19 and 30, and lysine at positions 1, 23, 32, 38 and 45) all occur on the surface of the molecule. The side chains of the polar amino acids (asparagine at position 11, glutamine at position 22 and threonine at position 41) also occur on the surface of the molecule. Furthermore, the hydrophobic amino acids, (such as the side chains of leucine at positions 8, 15, 24 and 33 and valine at position 18) are also solvent-accessible.
>
> The Three-dimensional modeling of the protein indicates that the arginine residue at position 10 is important to retention of the appropriate 3-dimensional structure and possible folding through hydrogen bond interactions with the C-terminal residue of the protein. A lysine, methionine or threonine substitution at that point would disrupt this hydrogen bonding network, leading to a destabilization of the structure. The synthetic peptide having this substitution could not be made to fold correctly, which supported this analysis. Conservation of the arginine residue at position 10 provides a protein which folds correctly.
>
> Alpha hordothionin has been modified to contain 12 lysine residues in the mature hordothionin peptide, referred to as HT12. (Rao *et al.* 1994

Protein Engineering 7(12):1485-1493 and WO 94/16078 published July 21, 1994) The disclosure of each of these is incorporated herein by reference in their entirety.

Further analysis of substitutions which would not alter the 3-dimensional structure of the molecule led to replacement of Asparagine-11, Glutamine-22 and Threonine-41 with lysine residues with virtually no steric hindrance. The resulting compound contains 27% lysine residues.

Other combinations of these substitutions were also made, including changes in amino acid residues at one or more of positions 5, 11, 17, 19, 22, 30 and 41 are lysine, and the remainder of the residues at those positions are the residues at the corresponding positions in the wild type hordothionin.

Numerous examples are provided in the specification of proteins appropriate in the invention. Included in such examples is the soybean 2S albumin described in U.S. Patent No. 5,850,016, and the chymotrypsin inhibitor from barley, Williamson et al. *Eur. J. Biochem* 165: 99-108 (1987). Specification page 12, lines 1-5. Production of derivatives of the genes through site directed mutagenesis is also described, and examples provided with the gene encoding barley high lysine polypeptide (BHL), derived from barley chymotrypsin inhibitor, citing PCT/US97/20441. Further, the gene encoding for enhanced soybean albumin gene (ESA) is cited as derived from soybean 2S albumin. Specification page 12, lines 6-14. In addition to an HT12 sequence, an ESA sequence was disclosed as SEQ ID NO: 6, appearing also at Table 2, page 40.

In an amendment of March 11, 2002 (responding to a written description rejection, but which is relevant here as well), in response to a request by the Examiner to point out references showing the availability of such proteins to one skilled in the art, the Applicant provided citations and copies of numerous references. Included among the references were: the 10kD zein storage protein

from maize, Kirihara et al. 1988, *Mol. Gen. Genet.* 211: 477-484; sulfur-rich 10kD rice prolamin, Masumura et al., *Plant Mol. Biol.* 12: 123-130, 1989; pea genes encoding high sulfur protein, Higgins et al., J. *Biol. Chem.*, vol. 261, No. 24, pp. 11124-11131 (1986); 12S seed storage protein gene from rapeseed, Ryan et al., *Nucleic Acids Res.*, 17 (9): 3584 (1989); sunflower 2S albumin gene, Allen et al., *Mol. Gen. Genet.*, 201(2): 211-218 (1987); maize albumin b-32 gene, Di Fonzo et al., *Mol. Gen. Genet.*, 212(3): 481-487 (1988); napin gene, Joseffson et al., *J. Biol. Chem.*, 262 (25): 12202-12208 (1987); B1 hordein gene, Forde et al., *Nucleic Acids Res.* 13(2)): 7327-7339 (1985); wheat alpha and beta gliadin genes, Sumner-Smith et al., *Nucleic Acids Res.*, 13(11): 3905-3916 (1985); wheat gliadin, Anderson et al., *Nucleic Acids Res.*, 12(21): 8129-8144 (1984); pea legumin gene, Lycett et al., *Nucleic Acids Res.*, 12(11): 4493-4506; various maize zeins, disclosed in Heidecker and Messing, *Nucleic Acids Res.*, 11(14): 4891-906 (1983); alpha, alpha', and beta-subunits of soybean 7S seed storage protein, Schuler et al., *Nucleic Acids Res.*, 10(24): 8225-8244 (1982); sunflower 11S gene, Vonder Haar et al., *Gene*, 74(2): 433-443 (1988); and pea convicilin gene, Bown et al., *Biochem. J.*, 251(3): 717-726 (1988). Other references were also provided; these are a sample of the various proteins one skilled has available for use with the invention.

Further, the declaration of Dr. Rudoph Jung of October 18, 1999, shows comparison of control plant lysine, cysteine and methionine content with plants expressing the HT12 sequence and ESA sequence, showing that most of the samples demonstrated at least a 10% increase, or at least a 15% increase, or at least a 20% increase in lysine and in sulfur containing amino acids.

Thus the Applicant submits that one skilled in the art, upon reading the specification, can readily produce an amino acid encoding sequence of the invention. Linking such a protein with an endosperm preferred promoter is likewise well within the abilities of one skilled in the art.

It is the burden of the Patent Office to set forth a reasonable explanation as to why it believes the scope of claim protection is not enabled by the specification. *In*

re Wright, 999 F.2d 1557, 1561-62 (Fed. Cir. 1993). Applicant submits that the Patent Office has not set out a *prima facie* case of non-enablement and any such demonstration has been rebutted by the Applicant.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

Claim 79 is to a transgenic cereal plant seed produced by the method of claim 113 and claim 114 is to a transgenic cereal plant seed comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid. In addition to the enabling disclosures pointed out in regard to claim 113, production of a transgenic plant seed is enabled disclosures which include page 23-27, Example 4 and Table 1, as well as the knowledge of one skilled in the art.

Claim 90

The claim is to an expression cassette according to claim 112 wherein the promoter is a gamma zein promoter or a waxy promoter. In addition to the enabling disclosures directed to claim 112, these are promoters known to one skilled in the art and citations for such well-known promoters are provided at page 18, lines 7-17 in the specification.

Claim 91 and Claim 112

These two claims are to a vector comprising the expression cassette of claim 112 (claim 91) and expression cassette comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine

or about 6 mole % to about 40 mole % of sulfur-containing amino acid (claim 112). In addition to the enabling disclosure and knowledge of one skilled in the art supporting claim 113, the specification provides at Table 2 at page 40 of the specification expression cassettes as examples.

Claim 105

The claim is to a transgenic cereal plant seed of claim 114 where the endosperm-preferred promoter is heterologous to the polynucleotide. In addition to the enabling support for claim 114, one skilled in the art can readily identify an endosperm-preferred promoter heterologous to the polynucleotide, and examples are provided in the specification at page 18, lines 1-18.

Claim 113

This claim is to a method for increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, which method comprises transforming the plant cell with an expression cassette which comprises a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, wherein the level of lysine or sulfur-containing amino acid is increased compared to a non-transgenic plant, and regenerating a plant to produce plant seed. Such a method is taught by the specification, which describes the methodology of producing a polynucleotide encoding such a barley alpha-hordothionine. See the specification at pages 2-3, 9-10 and 12-13, describing methods and alternatives, and Examples 1-4 which provide details for such a method. Specific examples are provided in the specification, and also in the declaration of Dr. Jung. Further, one skilled in the art would appreciate variations in the methodology.

Claim 115 and 116

A transgenic cereal plant (claim 115) or plant cells (claim 116) are claimed comprising an endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid. In addition to the knowledge of one skilled in the art, and the enabling disclosure for claim 113, further description of producing of plants and plant cells is provided in the specification at pages 21, 23-25, Example 4 and Table 1.

VIIIB ARGUMENTS – Rejections Under 35 U.S.C. § 112, Second Paragraph

Rejections of claims 79, 90, 91, 103, 105 and 112-116

Claims 112-116 and dependent claims 79, 90, 91, 103 and 105 are rejected as indefinite and confusing in the recitation of "one or more" with regard to a range of mole percent of lysine and of mole percent of a sulfur containing amino acid, since only two ranges appear. An amendment was submitted January 25, 2005 which amended claims 112-116 to recite "one or both" instead or "one or more." Claim 103 was cancelled in this amendment. Since the amendment complies with the Examiner's request, does not introduce new matter, and merely clarifies confusing language, it is requested that the amendment be entered pursuant to MPEP §1210.

Claims 115 and 116 and all claims dependent thereon are rejected as indefinite in that they are unduly alternative in the multiple recitations of "and/or" in the claims. Applicants point out that claims 115 and 116 were amended in an amendment of January 19, 2004 to remove "and" to recite only "or." Thus it is believed the rejection is moot.

VIIIC ARGUMENTS – REJECTIONS UNDER 35 U.S.C. § 102

Rejection of Claim 103

Claim 103 is rejected under 35 U.S.C. §102(b) as being anticipated by Powell et al , U.S. Patent No. 3,909,288. Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

VIIID ARGUMENTS – REJECTIONS UNDER 35 U.S.C. § 103

Rejection of Claims 79, 90, 91, 103, 105 and 112-116 as being obvious over Rao et al. U.S. Patent 5,885,802 "in view of Applicants' admission"

Claims 79, 90, 91, 103, 105 and 112-116 are rejected over Rao et al., U.S. Patent 5,885,802 "in view of Applicants' admission." The Examiner says that Rao's invention makes the invention obvious in that Rao is to "increasing amino acid composition of the seed (the major portion of which is the endosperm) with the constitutive promoter, and that one would have been motivated to substitute a seed-specific or endosperm-specific promoter to further increase or to limit increases in the seed/endosperm tissue." The admission cited is that endosperm-specific promoters are well known in the art.

Applicant agrees that endosperm-preferred promoters are well known in the art at the time of the invention. The claims are not directed to endosperm-preferred promoters *per se*, but rather to the unexpected result of the use of an endosperm-preferred promoter with a barley alpha-hordothionin protein, modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, in resulting in an increase in the level of lysine or sulfur-containing amino acids when compared to a non-transgenic cereal plant seed. The Rao reference uses a constitutive promoter, and does not teach or

suggest that one can expect improved results when using endosperm-preferred promoters. In fact, the reference teaches away from use of endosperm-preferred promoters, in the statement found at column 5, lines 7-9 that "the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency." There is no motivation to modify the Rao teachings to instead use an endosperm-preferred promoter. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference," *In re Kotzab*, 208 F.3d 1352 (Fed. Cir. 2000).

Such motivation is not found in the teachings, or in the observation that the endosperm is a major portion of the seed of the plant. That, alone, does not motivate one to chose an endosperm-*preferred* promoter to increase lysine and sulfur-containing amino acids in the content of the seed. Why not select a seed-preferred promoter? Then all the tissue will contain the expressed protein. Why not use the strong constitutive promoter of Rao and aim for high expression by use of a strong promoter expressing throughout the plant? No motivation or teaching of success is provided. Applicants submit a *prima facie* case of obviousness has not been presented.

Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

The Rao reference does not teach a transgenic cereal plant seed which has been produced comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain

one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid and increasing lysine or sulfur-containing amino acid compared to a non-transgenic cereal plant.

Claim 90

An expression cassette having a gamma zein or waxy promoter to obtain increased lysine and sulfur-containing amino acids produced from a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid is not taught in Rao.

Claim 91 and Claim 112

Neither a vector comprising the expression cassette of claim 112 (claim 91) nor the expression cassette of claim 112 with an endosperm-preferred promoter are taught by Rao.

Claim 105

The transgenic cereal plant seed of claim 114 having an endosperm-preferred promoter heterologous to the polynucleotide linked to the polynucleotide of the invention is not taught by Rao.

Claim 113

Rao does not teach a method of increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, comprising an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acid levels when compared to a non-transgenic plant.

Claim 115 and 116

The plants and plant cells of Rao do not use, and Rao does not teach an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acids compared to non-transgenic plants.

Rejection of Claims 79, 90, 91, 103, 105 and 112-116 as being obvious over Rao et al. U.S. Patent 5,990,389 "in view of Applicants' admission"

Claims 79, 90, 91, 103, 105 and 112-116 are rejected over Rao et al., U.S. Patent 5,990,389 "in view of Applicants' admission." The Examiner says that Rao's invention makes the invention obvious in that Rao is to "increasing amino acid composition of the seed (the major portion of which is the endosperm) with the constitutive promoter, and that one would have been motivated to substitute a seed-specific or endosperm-specific promoter to further increase or to limit increases in the seed/endosperm tissue." The admission cited is that endosperm-specific promoters are well known in the art.

Applicant agrees that endosperm-preferred promoters are well known in the art at the time of the invention. The claims are not directed to endosperm-preferred promoters *per se*, but rather to the unexpected result of the use of an endosperm-preferred promoter with a barley alpha-hordothionin protein, modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid, in resulting in an increase in the level of lysine or sulfur-containing amino acids when compared to a non-transgenic cereal plant seed. The Rao reference uses a constitutive promoter, and does not teach or suggest that one can expect improved results when using endosperm-preferred promoters. In fact, the reference teaches away from use of endosperm-preferred promoters, in the statement found at column 4, lines 65-67 that "the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency." There is no motivation to

modify the Rao teachings to instead use an endosperm-preferred promoter. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference," *In re Kotzab*, 208 F.3d 1352 (Fed. Cir. 2000).

Such motivation is not found in the teachings, or in the observation that the endosperm is a major portion of the seed of the plant. That, alone, does not motivate one to choose an endosperm-*preferred* promoter to increase lysine and sulfur-containing amino acids in the content of the seed. Why not select a seed-preferred promoter? Then all the tissue will contain the expressed protein. Why not use the strong constitutive promoter of Rao and aim for high expression by use of a strong promoter expressing throughout the plant? No motivation or teaching of success is provided. Applicants submit a *prima facie* case of obviousness has not been presented.

Claim 103 was cancelled in the amendment of January 25, 2005. Since the amendment complies with the Examiner's request, does not introduce new matter, and cancels the claim, it is requested that the amendment be entered pursuant to MPEP §1210.

Why Appellant Believes the Claims to be Separately Patentable:

Claim 79 and 114

The Rao reference does not teach a transgenic cereal plant seed which has been produced comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid and increasing lysine or sulfur-containing amino acid compared to a non-transgenic cereal plant.

Claim 90

An expression cassette having a gamma zein or waxy promoter to obtain increased lysine and sulfur-containing amino acids produced from a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of sulfur-containing amino acid is not taught in Rao.

Claim 91 and Claim 112

Neither a vector comprising the expression cassette of claim 112 (claim 91) nor the expression cassette of claim 112 with an endosperm-preferred promoter are taught by Rao.

Claim 105

The transgenic cereal plant seed of claim 114 having an endosperm-preferred promoter heterologous to the polynucleotide linked to the polynucleotide of the invention is not taught by Rao.

Claim 113

Rao does not teach a method of increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant, comprising an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acid levels when compared to a non-transgenic plant.

Claim 115 and 116

The plants and plant cells of Rao do not use, and Rao does not teach an endosperm-preferred promoter linked with the polynucleotide of the invention and resulting in increased lysine and sulfur-containing amino acids compared to non-transgenic plants.

IX APPENDIX OF CLAIMS

The text of the claims involved in the appeal are:

79. A transgenic cereal plant seed produced by the method of claim 113.

90. The expression cassette according to claim 112 wherein the promoter is a gamma zein promoter or a waxy promoter.

91. A vector comprising the expression cassette of claim 112.

105. The transgenic cereal plant seed of claim 114 wherein the seed endosperm-preferred promoter is heterologous to the polynucleotide.

112. An expression cassette comprising a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid.

113. A method for increasing the level of one or both of lysine or sulfur-containing amino acids in a cereal plant seed, the method comprising:

a) transforming a cereal plant cell with an expression cassette, and

b) regenerating a transgenic cereal plant to produce a transgenic cereal plant seed,

wherein the expression cassette comprises a seed endosperm-preferred promoter operably linked to a polynucleotide encoding a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein the level of lysine or sulfur-containing amino acid is increased in the transgenic cereal plant seed compared to a corresponding non-transgenic cereal plant seed.

114. A transgenic cereal plant seed comprising a modified barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein the transgenic cereal plant seed comprises an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

115. A transgenic cereal plant comprising a modified barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein

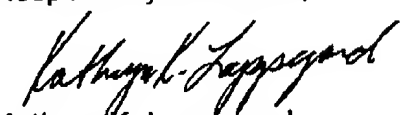
modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein transgenic seed of the transgenic cereal plant comprise an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

116. A transgenic cereal plant cell comprising a barley alpha-hordothionin polynucleotide operably linked to a seed endosperm-preferred promoter, wherein the polynucleotide encodes a barley alpha-hordothionin protein modified to contain one or both of about 7 mole % to about 40 mole % lysine or about 6 mole % to about 40 mole % of a sulfur-containing amino acid and wherein transgenic seed resulting from the transgenic plant cell comprise one or both of an elevated level of lysine or sulfur-containing amino acid compared to a corresponding non-transgenic cereal plant seed.

SUMMARY

In view of the above comments, overruling of the outstanding rejections and allowance of all the remaining claims is respectfully requested.

Respectfully submitted,



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